REMARKS

Status Summary

Claims 28, 29, 34-36, 41, 43, and 52 are pending. Claims 1 – 27, 30 – 33 and 37 – 40 were canceled previously. Claims 42, 44 – 51, and 53 – 59 were previously withdrawn. Claims 28, 29, 34 – 36, 41, 43 and 52 are rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Claims 28, 29, 34-36, 41, 43 and 52 are rejected under 35 U.S.C. §112, first paragraph as allegedly failing to comply with the enablement requirement. Reconsideration in view of the following remarks is respectfully requested.

Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 28, 29, 34-36, 41, 43 and 52 are currently rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. Specifically, the examiner alleges that one of ordinary skill in the art could not reasonably determine the metes and bounds of the claim in view of the phrase "correspond to" (e.g., whether a DNA sequence would "correspond to" SEQ ID NO: 12 if the sequence had a deletion at position 100 and a mutation at position 222). Official action, p. 2.

In reviewing a claim for compliance with 35 U.S.C. §112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. 112, second paragraph, by providing clear warning to others as to what constitutes infringement of the patent. *See*, e.g., *Solomon v. Kimberly-Clark Corp.*, 216 F.3d 1372, 1379, 55 USPQ2d 1279, 1283 (Fed. Cir. 2000).

It is respectfully pointed out that one of ordinary skill in the art is apprised of the scope of the claims because one of ordinary skill would understand that whether or not a particular DNA molecule that encoded a mature enzyme having protox activity had at least amino acid substitution "at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 as set forth in the alignment shown in Table 1" would depend on aligning the sequence in question with SEQ ID NO: 12 (e.g., as shown in Table 1). As of the filing date, sequence alignment programs such as the PileUp program (GCG package, University of Wisconsin) were readily available to assist the skilled artisan in making such a determination.

As the examiner will appreciate, sequence alignment programs take into account differences in sequence lengths (such as caused by a single base deletion) when making such comparisons. *See* e.g., Table 1A, pp. 120 – 122. As the instant claims provide clear warning to others as to what constitutes infringement of the patent, withdrawal of the rejection of claims 28, 29, 34-36, 41, 43 and 52 under 35 U.S.C. §112, second paragraph is respectfully requested.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 28, 29, 34-36, 41, 43 and 52 are rejected under 35 U.S.C. §112, first paragraph as allegedly lacking enablement. Specifically, while the examiner acknowledges that the instant application enables methods of transforming the plastome of a tobacco plant [with a DNA sequence comprising] a mutation at position 226 of SEQ ID NO: 12, the examiner alleges that that claims directed to plastome transformation in a plant other than tobacco are not enabled. Official action, p. 3. In support of this contention, the examiner cites Lutz, et al., (*Plant Physiology*, 2007, 145: 1201-1210). The examiner is apparently unpersuaded by the applicant's prior arguments absent further elaboration as to how the specification, the art described in the specification, and the teachings of the references previously cited by the examiner support the enablement of the entire scope of the claims. Official action, pp. 3 – 5.

In order to make an enablement rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). *See* MPEP 2164.04. Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention. *See* MPEP 2164.01. The standard by which this determination is made "is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.,* 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988), MPEP 2164.01.

A conclusion that a disclosure does not satisfy the enablement requirement and that any necessary experimentation is "undue" requires consideration of many factors, several of which

are listed in *In re Wands,* 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). These factors include, but are not limited to: (A) the breadth of the claims; (B) the nature of the invention; (C) the state of the prior art; (D) the level of one of ordinary skill; (E) the level of predictability in the art; (F) the amount of direction provided by the inventor; (G) the existence of working examples; and (H) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. *See* MPEP 2164.01(a). Initially, the applicants thank the examiner for reiterating the prior rejections.

(A) The breadth of the claims

Claim 28 is directed to a method for expressing a mature enzyme in a plant plastid comprising: (a) introducing into the plastome of a plant a chimeric gene comprising: (1) a modified DNA molecule that encodes a mature enzyme having protoporphyrinogen oxidase (protox) activity that is normally targeted to a plant plastid by a plastid transit peptide, wherein said DNA molecule is modified such that a coding sequence of the plastid transit peptide is absent from said modified DNA molecule and wherein said mature enzyme has at least one amino acid substitution to a naturally occurring protox enzyme that occurs at a position corresponding to position 221, 226, 227, 369, 371, 432, 436, 481, or 517 of SEQ ID NO: 12 as set forth in the alignment shown in Table 1, wherein said at least one amino acid substitution confers resistance to an inhibitor of said naturally occurring protox enzyme; and (2) a promoter capable of expressing said DNA molecule in a plastid, wherein said promoter is operatively linked to said DNA molecule, (b) expressing said DNA molecule in a plastid of said plant, wherein said mature enzyme is produced in said plastid. Claims 34 and 44 depend therefrom.

Claim 35 is directed to a method for expressing a mature enzyme in a plant plastid comprising: (a) introducing into the plastome of a plant a chimeric gene comprising: (1) a modified DNA molecule that encodes a polypeptide comprising: (i) a modified, non-functional plastid transit peptide, wherein said modified, non-functional transit peptide is not competent for import in a plastid, and (ii) a mature enzyme having protoporphyrinogen oxidase (protox) activity that is normally targeted to a plant plastid by a functional plastid transit peptide and wherein said mature enzyme has at least one amino acid substitution to a naturally occurring protox enzyme that occurs at a position corresponding to position 221, 226, 227, 369, 371, 432,

436, 481, or 517 of SEQ ID NO: 12 as set forth in the alignment shown in Table 1, wherein said at least one amino acid substitution confers resistance to an inhibitor of said naturally occurring protox enzyme; and (2) a promoter capable of expressing said DNA molecule in a plastid, wherein said promoter is operatively linked to said DNA molecule, and (b) expressing said DNA molecule in a plastid of said plant, wherein said polypeptide is produced in said plastid. Claims 36, 41 and 52 depend therefrom.

In the official action, the examiner has alleged that the breadth of the claims is overly broad. The applicants do not accede to the allegation that they are "overly" broad inasmuch as it conveys a position that the claims lack enablement.

(B) The nature of the invention

As the examiner notes, the present claims are related to a number of technologies and scientific concepts that are core to plant genetics and the genetic transformation of plants. Official action, p. 6. Specifically, the claimed inventions relate to the selection, delivery and expression of DNA molecules encoding mature enzymes having protox activity in plant plastids.

(C) The state of the prior art

As described in the applicants' prior response submitted, as of the earliest priority date, plastome transformation techniques had been described in the art.

Maliga et al (U.S. Patent No. 5,451,513) disclose and claim methods for obtaining a plant cell of a multicellular plant, the plastids of which cell have been stably transformed by a foreign DNA of interest, as well as transformed cells and multicellular plants, the plastids of which have been stably transformed by a foreign DNA of interest. Furthermore, Maliga et al teach that the plant cell may be a cell from any multicellular plant, for example cells of trees used for their timber or fruit, field plants such as plants grown for grain, or any other plants. Although Maliga et al only describe examples of the use of the claimed methods in tobacco, Maliga et al's methods are not limited to a particular plant species.

McBride et al (U.S. Patent No. 5,545,817) disclose and claim methods useful for genetic engineering of solanaceous plant cells to provide increased expression in the plastids of the plant

or plant cell. Although McBride et al only describe examples of the use of the claimed methods in tobacco, McBride et al's methods are not limited to just tobacco.

McBride et al (U.S. Patent No. 5,545,818) disclose and claim methods useful for genetic engineering of plant cells to provide increased expression in the plastids of a plant or plant cell of the *B. thuringiensis* insecticidal protein. McBride et al disclose that where transformation and regeneration methods have been adapted for a given plant species, either by *Agrobacterium*-mediated transformation, bombardment or some other method, the established techniques may be modified for use in selection and regeneration methods to produce plastid-transformed plants. For example, McBride et al provide that the methods described therein for tobacco are readily adaptable to other solanaceous species, such as tomato, petunia and potato. Although McBride et al only describe examples of the use of the claimed methods in tobacco, McBride et al's methods are not limited to a particular plant species.

McBride et al (U.S. Patent No. 5,576,198) disclose and claim methods useful for genetically engineering plant cells to control the timing or tissue pattern of expression of foreign DNA sequences inserted into the plant plastid genome, specifically the use of such methods in potato, corn, flowers such as petunia, rose, and carnation, fruits, such as tomato, and oilseed crops such as Brassica, soybean, corn, safflower, or sunflower. Although McBride et al only describe examples of the use of the claimed methods in tobacco, McBride et al's methods are not limited to a particular plant species.

Maliga et al (WO 97/32977) disclose and claim methods for the transformation of plastids in plants, specifically those from the *Cruciferae* family (e.g., *Arabidopsis*). Maliga et al demonstrate plastid transformation in *Arabidopsis* and *Brassica napus*, creating e.g., kanamycin and spectinomycin-resistant plants thereby.

All but one of the above prior art references are U.S. patents, which, as the examiner is aware, are entitled to a presumption of validity under 35 U.S.C. §282. While the examiner has previously stated the patentability of the claims of these patents is inapposite to the patentability of the instant claims, the applicants respectfully point out that the claimed inventions therein are entitled to the presumption that they are enabled (i.e., that each of the patents has an enabling disclosure). For example, even though although the '513 patent only provides examples of the use of the claimed methods in tobacco, it is presumed enabled for *any* plant. Therefore, there is a

presumption, which the examiner has not addressed, that practicing any of the claimed inventions described therein would *not* require undue experimentation.

(D) The level of one of ordinary skill

As the examiner notes, one of ordinary skill would be comfortable with the multidisciplinary nature of the claimed inventions, and would have a strong understanding of plant genetics and genetic transformation. One of ordinary skill in the art would also understand that the specification omits that which would be considered routine in the art. Official action, p. 6.

(E) The level of predictability in the art

The examiner alleges that as of the earliest priority date, there existed a number of challenges for genetically modifying the plastome in plants other than the tobacco plant. For example, the examiner states that Lutz et al teach that transformation of a plant on the plastome level was only routine for tobacco plants:

> *"For example, a typical Arabidopsis leaf cell contains approximately 120 chloroplasts and a total of 1,000 to 1,700 ptDNA copies (Zoschke et al., 2007) while an average tobacco (Nicotiana tabacum) leaf cell carries approximately 100 chloroplasts and approximately 10,000 ptDNA copies (Shaver et al., 2006). Transformation of the nuclear genome is routine in higher plants and is reviewed in this Focus Issue of Plant Physiology. Plastid transformation is routine only in tobacco (Svab et al., 1990; Svab and Maliga, 1993), but has rapidly expanded to diverse crops including potato (Solanum tuberosum; Sidorov et al., 1999), tomato (Solanum lycopersicum; Ruf et al., 2001), lettuce (Lactuca sativa; Lelivelt et al., 2005; Kanamoto et al., 2006), soybean (Glycine max; Dufourmantel et al., 2004), cotton (Gossypium hirsutum; Kumar et al., 2004), cauliflower (Brassica oleracea; Nugent et al., 2006), and poplar (Populus alba; Okumura et al., 2006). Transformation of mitochondrial DNA remains a challenge for the future."* Official action, pp. 7 – 8.

The examiner further supports these allegations by citing Kanevski et al. The examiner alleges that Kanevski teaches the transformation of a sunflower plant at the plastome level based on an understanding of the tobacco plant, but shows how technical obstacles arise to a successful

transformation, and include factors such as incompatibility at the level of translation, or protein folding. Official action, p. 8. However, contrary to what the examiner alleges, Kanevski et al examines the *transformation of tobacco plants* with the Rubisco gene *rbcL* from either sunflower or the cyanobacterium *Synechococcus* PCC6301. Therefore, any alleged difficulties as reported Kanevski et al cannot be applied as a general observation regarding the state of the art with respect to plastome transformation , because a) the examiner has acknowledged that plastome transformation in tobacco was known and enabled prior to the publication of Kanevski et al; b) Kanevski explained that "[w]estern analysis indicated that if [*Synechococcus* PCC6301] protein is produced it is transient and does not accumulate enough to be detected by antibodies raised against the cyanobacterial large subunit"; and c) Kanevski et al reported successful transformation of tobacco using the sunflower rbcL-S gene. *See* Kanevski et al., p. 139, left hand column.

The examiner appears to allege that, because one reference states that (as of 2007) plastid transformation in any plant other than tobacco was not "routine", and that difficulties were reported in one particular case, plastid transformation in any plant other than tobacco at the earliest priority date would have required experimentation that was beyond routine and would have required its own discovery. Official action, p. 5.

It is the applicants' position that whatever may be gleaned from Lutz et al and Kanevski et al with respect to the predictability of this technology as of the earliest priority date, the teachings of the instant application and other prior art discussed hereinabove sufficiently demonstrate that there was not a lack of predictability with respect to this technology such that a skilled artisan would have to engage in undue experimentation in order to practice the instant claims.

(F) The amount of direction provided by the inventor(s)

The instant application teaches plastome transformation of a wide range of monocot and dicot species with chimeric genes that express modified protox enzymes, such as those described in the instant claims. For example, the specification teaches that plastome transformation can be achieved by (1) introducing the modified protox gene into a plastome of a cell, which is operatively linked to a promoter capable of inducing expression in both green and non-green

chloroplasts, (2) expressing the encoded enzyme in the plastids of the plant cells and (3) selecting a cell that is resistant to a herbicidal compound that naturally inhibits the activity of the enzyme, whereby the resistant cell comprises transformed plastomes (*see* e.g., p. 70, lines 15-20). The inventors further provide examples of promoters that may be used to express the modified protox enzymes, plastome transformation vectors and examples of herbicides that may be used as selective agents (*see* e.g., pp. 66-67, bridging paragraph; pp. 59-66; p. 68, lines 23-26).

Still further, the instant application acknowledges the importance of employing appropriate selectable markers (*see* pp. 70-71, bridging paragraph). In particular, it is described that antibiotic resistant markers may be ineffective markers for plant transformation. For example, natural spectinomycin and streptomycin resistance in maize obviate the use of the bacterial *aadA* gene, since expression of this gene also results in spectinomycin or streptomycin resistance. The instant application teaches that preferred selectable markers for plastome transformation include (1) markers that are selectively transcribed in plastids but not in nucleus, (2) markers that are not dependent on photosynthetic competence or the presence of fully differentiated chloroplasts, and (3) markers having a level of resistance that is dependent on an adjustable external parameter, such as light (*see* p. 72, lines 3-9). For example, chimeric genes are useful selectable markers for plastome transformation, including for example, mutated EPSP synthase genes, or mutated ALS genes fused to a promoter capable of expression in plant plastids to select transplastomic plant cells using various herbicides (*see* e.g., pp. 69-70).

(G) The existence of working examples

As the examiner notes, Examples 1 – 8 are directed to the isolation of various plant protox genes, such as wheat, soybean, cotton and others (pp. 74 – 80). Examples 9-12 are directed to protox clone testing regarding herbicides (pp. 81 – 85). Examples 13 – 15 are directed to certain protox mutants, and Example 16 is direct to an $A\Delta T_{226}$ mutant of SEQ ID NO: 12. Examples 17 – 19 are related to other mutant protox enzymes. Examples 20 – 47 are directed to certain gene-based transformation components of the invention, including certain vectors and components such as promoters, but there is no working example of a plant having a genetically modified plastome that expresses a mutant protox in a plastid other than a tobacco

plant. Official action, p. 7. However, it is the applicants' position that working examples are not required in view of the state of the prior art, and that the working example cited by the examiner is just one of many that the applicants' could have provided to illustrate the practice of the broader claimed inventive concept.

(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

The examiner alleges that, based on Lutz et al and Kanevski et al, there were unresolved issues in the relevant art pertaining to the genetic transformation of a non-tobacco plastome, and the amount of non-routine experimentation required would be high. Accordingly, one of ordinary skill in the art would allegedly have to resort to undue experimentation. Official action, p. 8.

Initially, the applicants point out that, for reasons discussed hereinabove, Kanevski et al is irrelevant to any issues relating to the transformation of a non-tobacco plastome that may have existed as of the earliest priority date. Furthermore, the alleged teachings of Lutz et al are not inconsistent with the state of the art as of the earliest priority date. As previously discussed, there were multiple U.S. patents that existed in the prior art that taught methods of transforming a non-tobacco plastome. As a presumption of validity/enablement must be acknowledged with respect to those aforementioned U.S. patents, and those patents disclose and claim methods of transforming non-tobacco plants, it must be accepted that a skilled artisan could have transformed plastomes of non-tobacco plants as of the earliest priority date without having to engage in undue experimentation.

Even if one were to agree with the the examiner's allegations regarding Lutz et al (i.e., as of 2007, plastome transformation in non-tobacco plants was not routine), this should not be construed that achieving such transformation would require undue experimentation. The additional art cited within Lutz are also not inconsistent with the applicants' position. The fact that investigators did not report the actual plastome transformation of, e.g., potato, tomato, lettuce, soybean, cotton, cauliflower and poplar until years after the earliest priority date of the instant application does not mean that the instant claims or the inventions of the U.S. patents

described previously could not have been, or were not, practiced as of the priority date of the instant application.

In the absence of the need for a skilled artisan to engage in undue experimentation, claims 28, 29, 34-36, 41, 43 and 52 are enabled in compliance with 35 U.S.C. §112, first paragraph. Accordingly, withdrawal of the rejections of claims 28, 29, 34-36, 41, 43 and 52 under 35 U.S.C. §112, first paragraph is respectfully requested.

CONCLUSION

All rejections having been addressed, it is respectfully submitted that claims 28, 29, 34-36, 41, 43 and 52 are in condition for allowance. A notice to that effect is earnestly solicited.

If any points remain in issue, which may be best resolved through a personal or telephone interview, the examiner is kindly requested to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,
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